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16. ABSTRACT

Fathead minnows (Pimephales promelas) were exposed during a partial chronic toxicity study to endrin concentrations in the water or food, or both, for 300 days encompassing reproduction. Tissue residues were analyzed at preset intervals for first-generation fish, and were also determined for embryos, larvae at hatch, and 30-day progeny. Gas-chromatographic and liquid-scintillation techniques were used to monitor the contribution of endrin from each source. The food was clams that had accumulated 14C-endrin when exposed to an endrin water concentration similar to that to which the fish were exposed.

Higher endrin tissue residues were accumulated from the water than from food. Maximum concentration factors were 0.8 from the food and 13,000 from the water. Residues contributed by endrin in the food were additive to those from the water at all life stages. Endrin in the food (0.63 ppm) significantly reduced survival of the fathead minnows, and fish exposed to both endrin sources had lower survival than those exposed to either source alone. Endrin residues in embryos and larvae were highest and larval survival lowest for progeny of adults exposed to endrin in both food and water. Survival of 30-day progeny was significantly reduced at all test exposures (0.63 ppm in the food, water exposures of 0.14 and 0.25 ppb, and all combinations of food and water exposure).

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Toxicity to Fathead Minnows of Endrin in Food and Water

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Abstract. Fathead minnows *(Pimephales promelas)* were exposed during a partial chronic toxicity study to endrin concentrations in the water or food, or both, for 300 days encompassing reproduction. Tissue residues were analyzed at preset intervals for first-generation fish, and were also determined for embryos, larvae at hatch, and 30-day progeny. Gas-chromatographic and liquid-scintillation techniques were used to monitor the contribution of endrin from each source. The food was clams that had accumulated 14C-endrin when exposed to an endrin water concentration similar to that to which the fish were exposed.

Higher endrin tissue residues were accumulated from the water than from food. Maximum concentration factors were 0.8 from the food and 13,000 from the water. Residues contributed by endrin in the food were additive to those from the water at all life stages. Endrin in the food (0.63 ppm) significantly reduced survival of the fathead minnows, and fish exposed to both endrin sources had lower survival than those exposed to either source alone. Endrin residues in embryos and larvae were highest and larval survival lowest for progeny of adults exposed to endrin in both food and water. Survival of 30-day progeny was significantly reduced at all test exposures (0.63 ppm in the food, water exposures of 0.14 and 0.25 ppb, and all combinations of food and water exposure).

Numerous aquatic researchers have been concerned about the importance of the water or the food chain, or both, as sources for biological magnification of pesticides in the aquatic environment (Reinert 1967 and 1970, Chadwick and Brocksen 1969, Grzenda *et al.* 1970, Harrison *et al.* 1970, Macek and Korn 1970, Eberhardt *et al.* 1971, Hamelink *et al.* 1971, Johnson *et al.* 1971, Murphy 1971, Epifanio 1973, and Petrocelli *et al.* 1975). None of these authors, however, presented data relative to situations where both types of exposure are involved at threshold levels of chronic toxicity. Subsequently, Jarvinen *et al.* (1976) conducted a partial chronic toxicity exposure of fathead minnows *(Pimephales promelas)* to DDT in the food or water, or both. Presence of DDT in the diet significantly reduced fish survival and a combination of DDT in both diet and water caused higher tissue residues and resultant lower survival than exposure through only one or the other of those routes. They concluded that food as well as water sources of chemical exposure must be considered when toxicity tests are designed or the conclusions from such tests are evaluated and that more

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refined criteria must be established to determine the survival requirements for aquatic life.

This study is an attempt to determine whether the response of the fathead minnow to the pesticide endrin (1,2,3,4,10,10-hexachloro-6,7,-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanophthalene), which differs in persistence, breakdown products, and toxicity from DDT, is similar to that observed by Jarvinen *et al.* (1976). Objectives of the study were to determine the influence of a food *versus* a water source of endrin on bioaccumulation and toxicity, to determine if endrin exposure *via* food and water produces greater toxicity and bioaccumulation than exposure *via* either of these routes, and to determine endrin concentration factors for fathead minnows and freshwater clams.

Materials and Methods

The design of the fathead minnow partial chronic exposure was the same as that described by Jarvinen *et al.* (1976). A proportional diluter (Mount and Brungs 1967) was modified to deliver two test concentrations and a control; the low test concentration was 60% that of the high concentration. The toxicant was introduced from a saturator system (Veith and Comstock 1975). Nominal endrin test concentrations, selected on the basis of acute and preliminary partial chronic data, were 0.28 and 0.17 ppb, respectively.

Chopped and oven-dried clam tissue that was either clean or contaminated by 14C-endrin was used for the fish's food. Clam exposures were conducted in a flow-through system described by Jarvinen *et al.* (1976). The toxicant consisted of a 14C-endrin-acetone stock solution introduced into the exposure system by syringe for a nominal endrin concentration of 0.28 ppb, equivalent to the highest concentration tested. The species, source, and sample number of the clams exposed have been described by Jarvinen *et al.* (1976). Clams were exposed for 3 weeks, as preliminary studies indicated that this duration was necessary for them to achieve an equilibrium with the 0.28-ppb exposure concentration of 14C-endrin. After the 3-week exposure the clams were removed and prepared for fish food by the methods described by Jarvinen *et al.* (1976). The food supply was kept frozen, and a small portion was removed daily for feeding.

On August 20, 1975, 41-(±3)-day-old fathead minnows (n = 100) were randomly assigned to each of 12 test chambers. The 12 chambers were used to expose the fish through a reproductive period of their life cycle to duplicates of (1) a control (no endrin in food or water); (2) endrin-exposed food, but no endrin in the water; (3) unexposed food, but 0.17 ppb endrin in the water; (4) endrin-exposed food and 0.17 ppb endrin in the water; (5) unexposed food, but 0.28 ppb endrin in the water; and (6) endrin-exposed food and 0.28 ppb endrin in the water.

The fish were sampled to determine total body tissue residues at 7, 14, 28, 56, 112, 224, and terminally at 300 days' exposure. Fish were not fed for 24 hr before sampling. Samples consisted of 10 fish per duplicate test chamber, until terminally, when all remaining fish were sampled. Samples were placed in tared glass beakers, weighed, and frozen at −20° C until analyzed. Results were determined on a whole body wet-weight basis.

During the spawning period embryos in excess of the 50 required for hatchability studies were placed in tared glass vials, weighed, preserved with petroleum ether to prevent dehydration, and stored in a freezer at −20° C until analyzed. Individual test-chamber samples were composited to provide a minimum of three 0.3-g samples for residue analysis.

Larvae at hatch were transferred in groups of 40 each to larval chambers for 30-day growth, survival, and residue studies. Tissue-residue analyses were performed on one composite sample from each of the 12 test exposures. Larvae at hatch not saved for 30-day studies were weighed and grouped by the same method used for embryo-residue samples to provide a minimum of one 0.3-g sample from

each test exposure for residue analysis. Residue analyses on embryos, larvae at hatch, and 30-day progeny were performed on a whole body, wet-weight basis.

To simplify the presentation of results, test treatments are coded as follows:

Endrin exposure	*Coding*
Clean water, clean food (control)	C
Clean water, endrin food	F
0.17 ppb endrin water, clean food	0.17 W
0.17 ppb endrin water, endrin food	0.17 W + F
0.28 ppb endrin water, clean food	0.28 W
0.28 ppb endrin water, endrin food	0.28 W + F

Results from duplicate chambers were combined, and the data are expressed as the mean ± standard error (S.E.).

Test chambers were siphoned when necessary to remove uneaten food and were brushed and siphoned weekly. A constant 16-hr photoperiod was maintained. Daylight light intensity varied from 25 to 41 lumens at the surface of the fish chambers.

During the study, water temperatures were maintained between 23.5° and 26.0° C and were checked daily in all test chambers. Routine water chemistries were determined weekly by the methods described by the American Public Health Association *et al.* (1971). Dissolved oxygen levels were never lower than 5.1 ppm or higher than 8.8 ppm. Mean total hardness, acidity, and alkalinity were 48.8, 2.4, and 42.6 ppm, respectively; pH was between 7.1 and 7.9. Endrin 99% Analytical Reference Standard, supplied by the Velsicol Chemical Corporation, Chicago, Illinois, was used as the test toxicant. Endrin concentrations in the water were measured once a week. In each sample set, analyses were made on a duplicate and a spiked sample of control water. Endrin was extracted from the test water with hexane and analyzed by gas chromatography. Recovery from spiked control water samples averaged 94.5 ± 0.9% S.E. (n = 46). Determined endrin water concentrations in the test exposures are presented in Table 1.

Endrin-1,2,4,10-14C with a specific activity of 1-5 mCi/m*M*, purchased in 50-μCi lots from California Bionuclear Corporation, Sun Valley, California, was dissolved in 50 ml of acetone. The 14C-endrin stock solution was prepared by dissolving a calculated gram weight of endrin (99% Analytical Reference Standard) in acetone to which 45 ml of the 14C-labeled endrin was added. Total volume was brought to 250 ml with acetone and the solution was thoroughly mixed. Only enough 14C-labeled endrin to give readable scintillation counts was combined with the unlabeled endrin. This procedure insured that the same stock of unlabeled material was the major source of endrin used in the fish and clam exposures. The clam 14C-endrin water concentrations averaged 0.24 ± 0.01 ppb

Table 1. Endrin water concentrations in exposure chambers

Nominal endrin water concentration (ppb)	Measured concentration (ppb)	
	N	Mean
C	43	0.00 (0.00)[a]
F	43	0.00[b] (0.00)
0.17 W	42	0.14 (0.00)
0.17 W + F	44	0.14 (0.00)
0.28 W	44	0.25 (0.01)
0.28 W + F	44	0.25 (0.00)

[a] Standard error in parenthesis
[b] Trace (<0.02 ppb)

S.E. (n = 6). Recovery of spiked control water samples averaged 91.5 ± 0.7% S.E. (n = 3). Water analysis for endrin was performed as described for the fish exposure.

Total tissue residues were determined by gas chromatography. Adult fish and 30-day progeny were extracted with a semi-micro blender using anhydrous Na_2SO_4 to dry the tissues and insure homogenization. Samples were blended at high speed and extracted with 15% ethyl ether in petroleum ether for 2 min. The solvent extract was decanted onto an anhydrous Na_2SO_4 column and collected in a tared beaker for lipid determination. Each sample was extracted four times. The solvents were evaporated and the lipid weight was determined. Lipid was redissolved in petroleum ether and the endrin was then cleaned up on a 20-g Florisil column as described by U.S. Department of Health, Education and Welfare (1971). Samples were eluted with 150 ml of 6% ethyl ether in petroleum ether and 200 ml of 15% ethyl ether in petroleum ether, and then concentrated to a volume of less than 10 ml on a steam bath. Analysis was completed by gas chromatography. Samples of embryos, larvae at hatch, and clam tissue were prepared and analyzed by the methods described by Jarvinen *et al.* (1976).

Radiometric methods were used to determine endrin residues derived from the food for all residue samples. Analysis was performed on a Packard Tri-Carb Liquid Scintillation Spectrometer. Sample preparation was that described by Jarvinen *et al.* (1976). A correlation was made between the gas chromatograms and scintillation counts through a series of dilutions of the 14C-endrin stock solution. An average count per min/μg of endrin was then calculated and used for the determination of the amount of endrin derived from the contaminated food. Before final calculations were made individual sample counts were corrected for background radiation and sample volume removed for gas chromatographic analysis.

Liquid scintillation and gas chromatographic results were not identical as they should have been for fish exposed to 14C-endrin-contaminated food only; liquid scintillation values were less than 50% of gas-chromatographic values. Analysis of the original 14C-labeled endrin indicated that only about 42% of the expected amount of endrin was present; the other 58% consisted of degradation products of which the keto form of endrin predominated. The keto form would be carried through extraction but not through Florisil; thus, approximately 58% of the 14C-tracer was lost. Accordingly, data for the residue samples analyzed for each time period were adjusted by the formula: adjusted liquid scintillation value = liquid scintillation value obtained/ratio liquid scintillation value to gas chromatographic value for fish exposed to 14C-endrin-contaminated food only.

All data on survival, embryo hatchability, percentage of lipid, and growth of 30-day-old progeny were subjected to one- or two-way analysis of variance to determine the endrin effect from food or water exposure. Dunnett's procedure (Steel and Torrie 1960) was used for comparison of treatment with control means.

Results

The 14C-endrin content of exposed clam tissue was 0.63 ± 0.01 ppm S.E. (n = 6) as determined by gas chromatography. This value indicates a concentration factor of approximately 3,000 based on the average measured endrin concentration in water of 0.24 ppb.

An apparent steady state with dietary endrin probably occurred within 14 days (Figure 1). Figures 2 and 3 show the additive residue effect of dietary endrin, compared to residues from a water source alone. Residue levels after 14 days of exposure were essentially greater in fish exposed to both dietary and water sources of endrin than for those exposed through the water only.

Use of the 14C label in the contaminated food allowed the use of liquid-scintillation analysis to separate endrin residues obtained through the food from the total endrin residues that were determined by gas chromatographic analysis. These data are shown in Table 2 and are expressed as a percentage of the total residues as determined by gas chromatographic analysis. The relative amount of

Table 2. Percentage of endrin residues attributable to the 14C-endrin food source after various periods of exposure (values shown are the means for samples from duplicate test chambers)

Nominal endrin water concentration (ppb)	Days of exposure							Mean for entire period
	7 (n = 2)[a]	14 (n = 2)	28 (n = 2)	56 (n = 2)	112 (n = 2)	224 (n = 2)	300 (n = 4)	
F	100[b] (34.5)[c]	100 (65)	100 (70.5)	100 (3.9)	100 (21.3)	100 (7.0)	100[d] (3.6)	100 (8.0) (n = 15)
0.17 W + F	15 (3.7)	15 (2.5)	10 (2.5)	10 (0.9)	18 (0.0)	19 (5.0)	18 (6.3)	15 (1.8) (n = 16)
0.28 W + F	8 (0.3)	8 (0.5)	6 (1.0)	8 (0.5)	14 (0.5)	12 (1.0)	14[e] (0.0)	10 (0.9) (n = 13)

[a] Number of residues samples
[b] Liquid-scintillation values shown are adjusted (see "Materials and Methods")
[c] Standard error
[d] n = 3
[e] n = 1

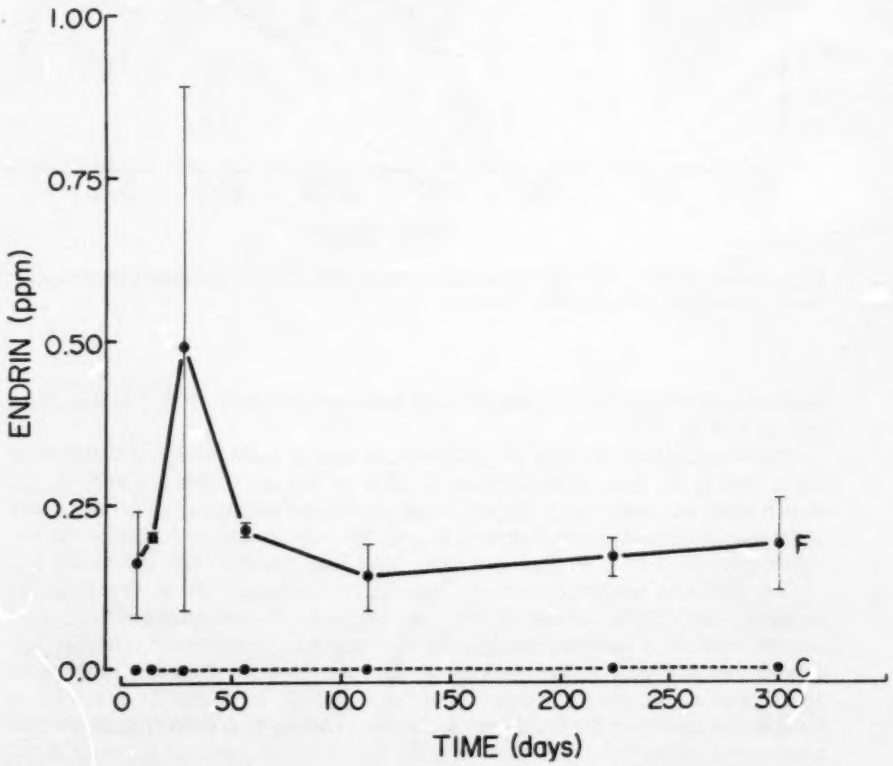

Fig. 1. Endrin residues in the controls and fish exposed to endrin in the food (vertical lines indicate standard error)

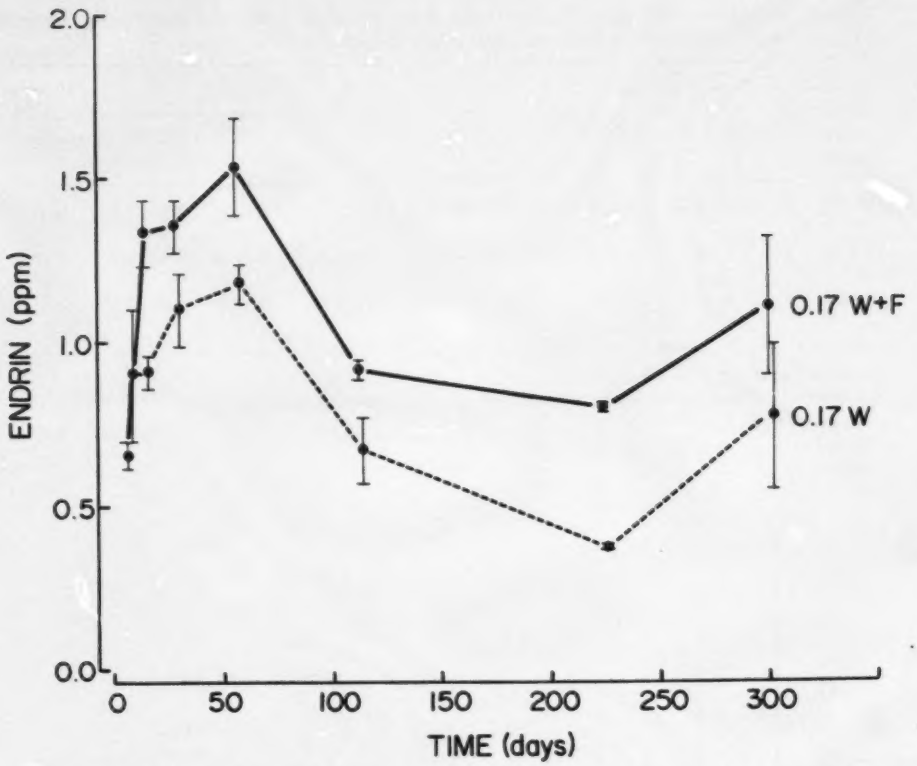

Fig. 2. Endrin residues in fish exposed to endrin in the water (0.17 ppb) or in combination of food and water (vertical lines indicate standard error)

residues contributed by the contaminated food was 15% at 0.17 W + F and about 10% at 0.28 W + F.

Mean calculated accumulation factors for endrin from food and water were 0.3 ± 0.01 S. E. for endrin from the food (n = 21) and 7,000 ± 436 S. E. for endrin from the water (n = 28, all water exposures combined). The maximum water concentration factor observed was 13,000, which occurred after 56 days of exposure at 0.28 W + F. The maximum food concentration factor was 0.8.

Survival was analyzed over the different sample periods as described by Jarvinen *et al.* (1976). To test whether the addition of endrin-contaminated food altered survival, a two-way analysis of variance was performed on the survival data shown in Figure 4. The hypothesis that the presence of endrin in the food does not affect survival was rejected at the P = 0.005 level. Survival was lower for fish fed endrin in the food than for those exposed to a corresponding water concentration but fed clean food (Figure 4). Although survival was not much different during the first 50 days between fish exposed to endrin in the food and

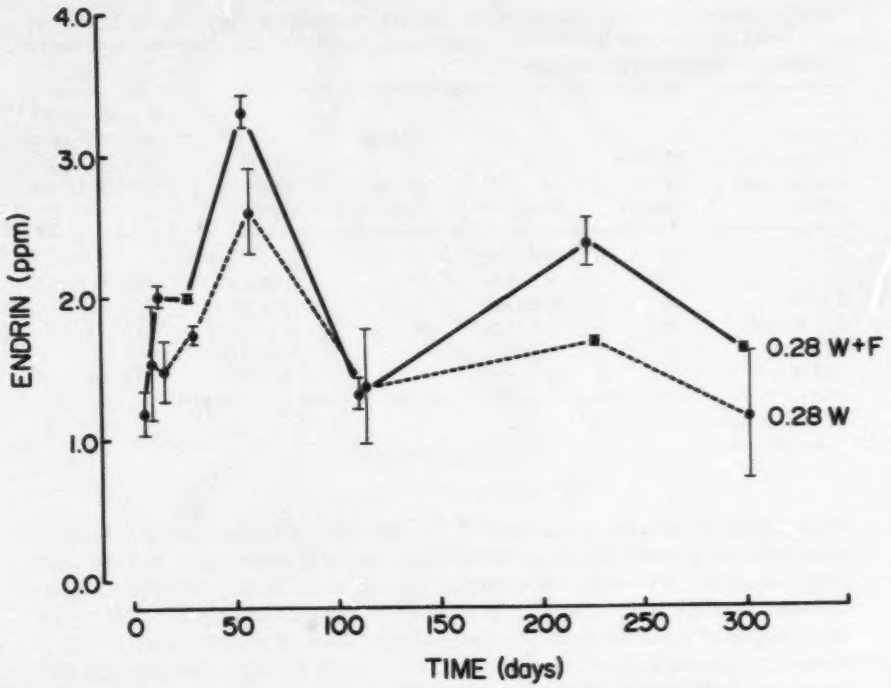

Fig. 3. Endrin residues in fish exposed to endrin in the water (0.28 ppb) or in combination of food and water (vertical lines indicate standard error)

those fed clean food, this difference became pronounced when the fish commenced spawning (112 days).

Embryo hatchability, residues, and percentage endrin derived from the contaminated food of the parent fish are presented in Table 3. No significant reduction in hatchability was demonstrated at any exposure level. Percentage endrin in embryos that was derived from contaminated food fed to the adult fish was similar to that found in the adult fish. The additive nature of residues from the contaminated food is indicated.

Residues in larvae at hatch and percentage residues caused by the contaminated food of the parent fish are presented in Table 4. Residues from contaminated food are again additive. With the exception of the F and 0.28 W + F exposures, there is about a 70% increase in residues above those found in the embryos; the increase at 0.28 W + F is 30%. The increase in residues was derived from water exposure because endrin derived from the contaminated food fed to the parent fish remained constant at about 0.05 ppm. Larvae were removed for analysis daily, so it is possible that some may have been exposed to endrin in the water for as long as 24 hr.

Residue data and percentage residues caused by the contaminated food for

Table 3. Hatchability, endrin residues (ppm), and percentage endrin contributed by the contaminated food in embryos from fish exposed to various test conditions (values shown are the means for samples from duplicate test chambers)

Nominal endrin water concentration (ppb)	Number of spawns	Hatch (%)	Number of residues samples	Endrin residues (ppm)	Endrin residues derived from contaminated diet of adult fish (%)
C	21	59.0 (4.6)[a]	8	—[b]	—
F	34	54.3 (3.9)	7	0.05 (0.01)	100 (11.5)
0.17 W	45	51.9 (3.8)	5	0.34 (0.01)	—
0.17 W + F	46	51.4 (3.0)	8	0.37 (0.03)	16.5 (2.1)
0.28 W	52	57.5 (3.6)	8	0.50 (0.03)	—
0.28 W + F	63	42.8 (4.0)	8	0.67 (0.04)	10.4 (2.8)

[a] Standard error
[b] Not detected

30-day progeny are shown in Table 5. No residues were detectable in progeny from exposed parents that were hatched and raised in clean water and fed clean food. Residues for some unexplained reason were lower in those progeny exposed through both routes than in those exposed through the water only. Percentages of residues from the contaminated food in food-exposed progeny, however, indicate its additive effect. Lower residues perhaps could be related to the lower mean weight of the progeny exposed to endrin in both the food and water (Table 6). None of these weight differences, however, are statistically significant. Table 6 also shows the percentage survival of the 30-day progeny. Survival was significantly lower for all test exposures than that for the controls.

Table 4. Endrin residues (ppm) and percentage endrin contributed by the contaminated food in larvae at hatch from fish exposed to various test conditions (values shown are the means for samples from duplicate test chambers)

Nominal endrin water concentration (ppb)	Number of samples	Endrin residues (ppm)	Endrin residues derived from contaminated diet of adult fish (%)
C	1	—[a]	—
F	2	0.05 (0.02)[b]	100 (13.5)
0.17 W	2	0.58 (0.01)	—
0.17 W + F	3	0.62 (0.04)	9.0 (0.5)
0.28 W	7	0.83 (0.02)	—
0.28 W + F	4	0.87 (0.03)	6.8 (0.2)

[a] Not detected
[b] Standard error

Table 5. Endrin residues (ppm) and percentage endrin contributed by the contaminated food in 30-day progeny from fish exposed to various test conditions (progeny exposed to same conditions as parent fish, values shown are the means for samples from duplicate test chambers)

Nominal endrin water concentration (ppb)	Number of samples	Endrin residues (ppm)	Endrin residues derived from contaminated diet of progeny and adult fish (%)
C	2	—[a]	—
F	2	0.24 (0.11)[b]	100 (24.0)
0.17 W	2	0.77 (0.05)	—
0.17 W + F	2	0.64 (0.02)	30.4 (15.6)
0.28 W	2	1.15 (0.05)	—
0.28 W + F	2	0.96 (0.05)	9.3 (2.2)
0.28 W[c]	2	—	—

[a] Not detected
[b] Standard error
[c] Progeny hatched and raised in clean water and fed clean food

Table 6. Growth and survival of 30-day progeny of fish exposed to various test conditions (progeny exposed to same conditions as parent fish, values shown are the means for samples from duplicate test chambers)

Nominal endrin water concentration (ppb)	Number of samples	Mean weight/fish (g)	Survival (%)
C	6	0.06 (0.01)[a]	50.1 (6.5)
F	8	0.07 (0.04)	10.9[b] (5.5)
0.17 W	6	0.05 (0.01)	15.0[b] (5.5)
0.17 W + F	6	0.04 (0.01)	10.8[b] (4.4)
0.28 W	6	0.05 (0.02)	7.4[b] (5.1)
0.28 W + F	7	0.02 (0.00)	3.7[b] (1.1)
0.28 W[c]	4	0.12 (0.04)	1.9[b] (1.1)
0.28 W + F[c]	2	—[d]	0.0[b]

[a] Standard error
[b] Values significantly different from the control
[c] Progeny hatched and raised in clean water and fed clean food
[d] No survivors

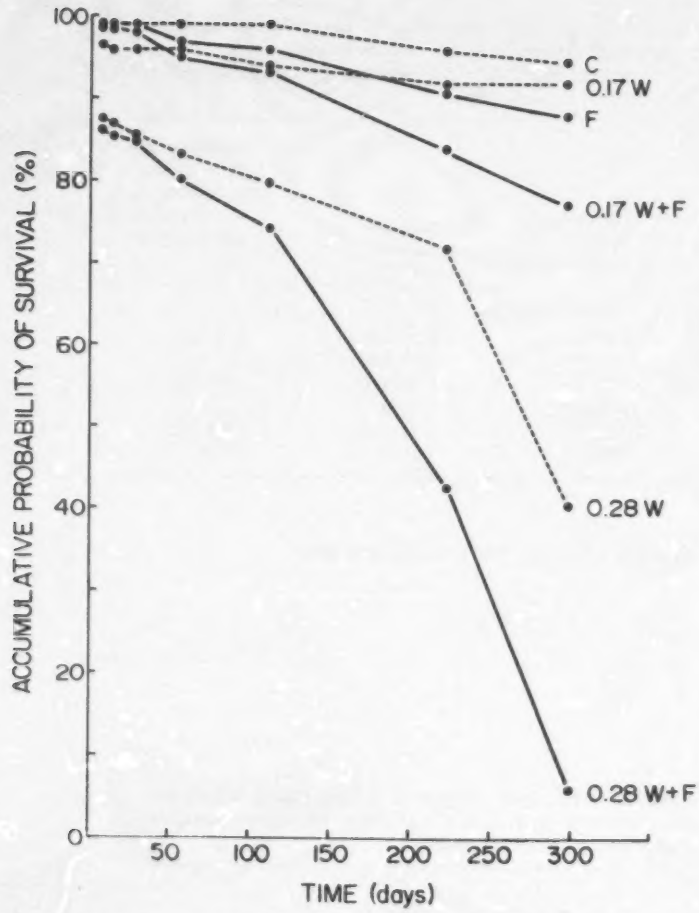

Fig. 4. Accumulative probability of survival for fish that had been exposed to endrin

Survival was also lower for all progeny fed dietary endrin than for progeny exposed only to endrin in the water.

Discussion

The endrin residue level in contaminated clam tissue (0.6 ppm) was similar to concentrations that have previously been demonstrated to occur in aquatic organisms in the environment. Residues as high as 0.5 ppm have been found in wild, pesticide-resistant mosquito fish *(Gambusia affinis)* (Finley *et al.* 1970),

and Jackson (1976) has stated that fish residing in agricultural areas frequently contain endrin residues exceeding 0.3 ppm.

Our results indicate that the uptake of endrin from the food was additive to that accumulated from the water and that an apparent steady state with the food was reached in approximately 14 days. These findings are similar to those obtained in a previous study with DDT (Jarvinen *et al.* 1976).

When fish were exposed to endrin in both food and water, body residues attributable solely to the diet comprised only 10 to 15% of the total endrin. Thus the main source of pesticide uptake was the water. This finding is consistent with other published results (Reinert 1967, Chadwick and Brocksen 1969, Jarvinen *et al.* 1976).

Our mean endrin concentration factors were 0.3 for dietary endrin and 7,000 for endrin from the water. These values are similar to those observed by other workers. Petrocelli *et al.* (1975) fed dieldrin-exposed clams to blue crabs, *(Callinectes sapidus),* and from their data a food concentration factor as high as 0.5 can be calculated. Jackson (1976) fed an endrin-contaminated diet (2.5 ppm) to channel catfish *(Ictalurus punctatus)* for 147 days and observed an average whole-body residue of 0.49 ppm, which indicates a food concentration factor of 0.2. Argyle *et al.* (1973) observed food concentration factors from 0.08 to 0.2 in fingerling channel catfish offered diets containing 0.04 to 4.0 ppm of endrin for 198 days. Johnson (1967) studied the effects of endrin on the medaka *(Oryzias latipes)* and calculated accumulation factors from the water of 17,000 to 26,000. Mount and Putnicki (1966) exposed fathead minnows to 0.015 ppb of endrin in the water and observed a concentration factor based on total body residues of 10,000. Residue accumulation up to 15,000-fold was observed in flagfish *(Jordanella floridae)* exposed for 60 days at 0.05 to 0.3 ppb of endrin in the water (Hermanutz 1978). Our highest observed concentration factor for endrin from the water was 13,000.

The presence of endrin in the diet significantly reduced the survival of the test fish. Deaths occurred in two definite periods—the first 7 days and the spawning period. These results are similar to those observed by Jarvinen *et al.* (1976) for fathead minnows exposed to DDT in food or water or both. Our survival data indicate that the toxic effects of endrin are cumulative, which is in agreement with the work of other researchers (Henderson *et al.* 1959, Jensen and Gaufin 1966, Johnson 1967). Before death our fish exhibited loss of equilibrium and lateral body rolling. These behavioral effects are similar to those described by Mount (1962) for endrin-exposed bluntnose minnows *(Pimephales notatus).*

Endrin in food or water, or both, did not significantly affect embryo hatchability, but survival of progeny was significantly reduced at all test exposures. Survival among progeny was lower than that observed for parent fish, and this indicates that continuous exposure to aqueous endrin concentrations of 0.14 ppb and higher would probably be detrimental to second-generation fish. This lower survival may have been caused by residues contributed from the parent fish. Generally, larvae of fish have been observed to be a very sensitive life stage (Holden 1973, McKim 1977).

Residues from endrin-contaminated food were additive to those from water exposure at all life stages. Even though endrin concentration may be greater from the water, presence of dietary endrin caused higher tissue residues and lower

survival than a corresponding water exposure alone. This study as well as an earlier one with DDT (Jarvinen *et al.* 1976) indicates that pesticide uptake through the food chain can have harmful effects on aquatic life. The addition of DDT through the food caused a 50% increase in toxicity (Jarvinen *et al.* 1976). In our study, dietary endrin increased toxicity 80%. Possibly the more toxic the pesticide is, the more important its incorporation *via* the food chain becomes. We can expect similar results with other pesticides and perhaps even other toxic substances. Consequently, possible food-chain effects should be carefully considered when developing water-quality criteria necessary for the survival of aquatic life.

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